



Supplementary Figure 1. Characterization of sstFRET. (A) Urea unfolding of the linker of purified sstFRET. The sstFRET protein was diluted in 200 μ l of Tris-HCl, 7.4, 1 M, 2 M, 4 M and 8 M urea. The spectrometer spectra scans were performed with 433 nm donor excitation and 450-600 nm emission. (B) Trypsin digestion of the linker. One unit of trypsin was used to cleave 100 nanomoles sstFRET protein in 200 μ l total volume. The scan used the same parameters set for urea unfolding. Data were acquired after 20 sec, 1 min and 2 min. (C) Temperature increased energy transfer of sstFRET. We then let the solution return to room temperature. (D) Trypsin digestion of purified Venus, acceptor. (E) Trypsin digestion of purified Cerulean, donor. All spectra of sstFRET were arbitrarily normalized to donor emission peak at 475 nm. All experiments were repeated at least three times.